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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of William D. Picking et al. Art Unit 1642
Serial No. 09/830,026
Filed October 20, 2001
Confirmation No. 9340
For METHOD FOR THE PRODUCTION OF PURIFIED INVASIN PROTEIN AND USE THEREOF
Examiner S. Devi

June 30, 2004

PETITION UNDER 37 CFR 1.181
TO WITHDRAW HOLDING OF ABANDONMENT

TO THE DIRECTOR OF THE UNITED STATES PATENT AND TRADEMARK OFFICE,

SIR:

In response to the Notice of Abandonment dated June 18, 2004, Applicants hereby respectfully petition to obtain withdrawal of the holding of abandonment.

The Notice of Abandonment states that Applicants failed to timely file a proper reply to the Office letter mailed on November 21, 2003.

In response to the Office action mailed November 21, 2003, applicants filed by Express Mail (EV 416452675 US) Amendment C, dated May 21, 2004; a Statement Under 37 C.F.R. 1.821(f); a computer readable and written sequence listing; an Information Disclosure Statement; and a check in the amount of $180.00. Copies of each of these papers are attached hereto for consideration. *

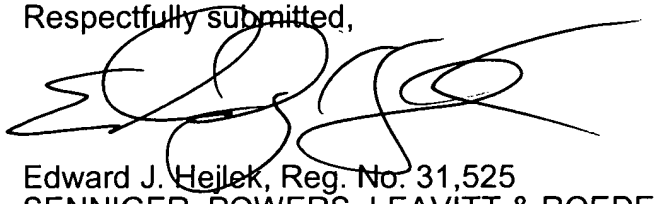
Evidence supporting a filing date of May 21, 2004, and delivery of these papers to the Patent Office includes: the Express Mail Receipt No. EV 416452675 US (which shows a "date in" of May 21, 2004), and a US Postal Service Track Record indicating acceptance on May 21, 2004 and delivery to the Patent Office on May 24, 2004 (item signed for by M. Boston). In addition, the return post card date-stamped by the Patent and Trademark Office as received on May 21, 2004, verifies the May 21, 2004 filing date and receipt by the Patent Office of Amendment C; sequence listing, certification, and diskettes; Information Disclosure Statement (including one reference); and check in the amount of $180.00. Copies of the Express Mail Receipt, US Postal Service Track Record, and return post card are enclosed. *

The present Petition Under 37 CFR 1.181 to Withdraw Holding of Abandonment is being filed less than two months after learning of the Notice of Abandonment.

In view of the foregoing remarks and attachments hereto, applicants respectfully submit that the Notice of Abandonment mailed June 18, 2004 was issued due to an error on the part of the Patent and Trademark Office and respectfully request withdrawal of the holding of abandonment. Applicants further request that the Office enter Amendment C and the accompanying papers, and that Amendment C be accorded its actual filing date of May 21, 2004.

Applicants believe that no fee is necessary in connection with this Petition; see MPEP §711.03(c). However, in the event that a petition fee is deemed necessary, Applicants respectfully request that such fee be charged to Deposit Account No. 19-1345.

Respectfully submitted,



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Client Name University of Kansas

Inventor(s) or Mark William D. Picking et al.

Serial No. 09/830,026 Patent/Reg. No. _____

The following has been received by the U.S. Patent and Trademark Office on the date stamped hereon:

- ☑ Amendment C
- ☐ Preliminary Amendment
- ☐ Response to Missing Parts Notice
- ☐ Assignment and Cover Sheet
- ☐ _____ Brief
- ☑ Check $ 180.00
- ☑ Letter to Commissioner
- ☐ RCE Transmittal
- ☐ Maintenance Fee
- ☐ Section _____ Declaration
- ☐ Fee Transmittal Form
- ☐ Combined Declaration/Power of Attorney
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- ☐ Status Inquiry
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- ☐ _____ Sheet(s) of Formal Drawing(s)
- ☐ Letter to Official Draftsman
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- ENROUTE, May 21, 2004, 8:02 pm, SAINT LOUIS, MO 63145
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of William D. Picking, et al. Art Unit 1642
Serial No. 09/830,026
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Examiner S. Devi

May 21, 2004

AMENDMENT C

TO THE ASSISTANT COMMISSIONER FOR PATENTS,

SIR:

In response to the Office Action dated November 21, 2003, please enter the following amendments:

Amendments to the Specification begin on page 2 of this paper.

Amendments to the Claims are reflected in the listing of claims which begins on page 7 of this paper.

Remarks begin on page 14 of this paper.

AMENDMENTS TO THE SPECIFICATION

Please replace the paragraph beginning on page 33, line 26 with the following:

Plasmid construction.

Isolation of plasmid DNA and all other molecular biology procedures were carried out according to standard published procedures. To confirm correct insertion of the desired fragments, plasmids were subjected to double-stranded DNA sequencing ([Sequenase] SEQUENASE 2.0) according to the manufacturer's specifications. PCR primers to the 5' and 3' ends of the *Ipa*C or *Sip*C DNA sequences were produced based on their published sequences. Each 5' primer contained the sequence GAGA (SEQ ID NO: 3), an NdeI restriction site and 18 bases of the 5' end of each gene, respectively. Each 3' primer contained GAGA (SEQ ID NO: 3), a BamHI restriction site and 18 bases of the 3' end of each gene, respectively. Each sequence was amplified by PCR in a standard 100 µl reaction containing 2.5 mM $MgCl_2$, 0.25 mM of each dNTP, 100 pmol of the 5' and 3' primers, 10 µl boiled *S. flexneri* or *S. typhimurium*, and 5 U Taq DNA polymerase. Reactions were allowed to proceed in a Perkin-Elmer 480 thermal cycler programmed for 29 cycles (94° C, 45 sec; 63° C, 30 sec; and 72° C, 60 sec) with one additional cycle for 10 min at 72° C. Upon establishing that each PCR product was of the correct size by agarose gel electrophoresis, 7 µl of the reaction mixture was used directly for ligation of the fragment into the pCRII plasmid (Invitrogen, Inc., San Diego, CA) according to manufacturers specifications. The plasmids were then transformed into *E. coli* INVaF' and the transformants containing inserts identified by blue-white screening. The presence of the specific *Ipa*C (SEQ ID NO. 2) or *Sip*C (SEQ ID NO. 1) gene fragments was then confirmed by PCR using the conditions described above (except that 25 µl reactions were used with a T7 promoter forward primer and M13 reverse primer).

Please replace the paragraph beginning on page 36, line 12 with the following:

SDS-PAGE and Western blot analysis.

SDS-PAGE was performed using the standard procedure of Laemmli, *Nature* 227:680, 1970. Following electrophoresis on 9% polyacrylamide gels, the samples could be stained with [Coomassie] COOMASSIE brilliant blue R250 or the proteins electroblotted to PVDF membranes (MSI, Westborough, MA) for Western blot analysis using a [BioRad] BIORAD Transblot Semi-dry Blotter according to the manufacturer's instructions . Western blot analysis was performed. Briefly, the membranes were blocked following protein transfer by incubation in nonfat dry milk in TBS (10 mM Tris-HCl pH 7.5, 150 mM NaCl) and then incubated with anti-SipC polyclonal antibodies or anti-IpaC monoclonal antibodies diluted in TBS containing 1 mM EDTA and 1% NP-40 (*v/v*). After several rinses in the same buffer, the membrane was incubated in ^{125}I-labeled protein G (100,000 dpm/ml) in the same buffer. The membrane was then rinsed in TBS containing 1 mM EDTA, 1 M NaCl and 0.4% N-laurylsarcosine (*w/v*), wrapped in plastic wrap, and exposed to Fuji X-ray medical film.

Please replace the paragraph beginning on page 27, line 10 with the following:

After allowing the production of the recombinant invasin protein in the host cells, the protein is purified according to a protocol appropriate for the affinity purification moiety employed in the method of the present invention, with the modification that all reagent solutions contain a protein denaturant. As the invasin adjuvant should be soluble in the cytosol of the host cell, or in the culture media if secreted, the supernatant should be used in the purification process once the cells or cell lysis debris have been pelleted by centrifugation. At this point, the denaturant should be added to

the protein solution to an appropriate concentration. Preferred denaturants for use in the present invention include guanidine hydrochloride and urea. Although surfactants such as [Tween] TWEEN and [Triton] TRITON may be used in the present invention, they are not preferred because of their tendency to form micelles, which are difficult to remove completely. The most preferable denaturant for use in the present invention is urea because of its efficacy as a denaturant and relatively low toxicity. The appropriate concentration for the denaturant in the protein solution is that concentration which will inhibit protein-protein interactions. For urea, this concentration is preferably between about 1 M and about 10 M, more preferably between about 5 M and about 7 M, and most preferably about 6 M. All solutions used in the purification process most preferably contain a denaturant at an appropriate concentration.

Please replace the paragraph beginning on page 44, line 21 with the following:

ELISA Assay

An ELISA assay was used to measure the levels of IgG subclasses to ovalbumin following immunization. The amount of ovalbumin used in the assay to coat the assay wells was 1 µg/well. Primary antibodies from the blood samples obtained are diluted 1:360 in 2% casein and are incubated with the ovalbumin antigen for 4 hours. After washing in PBS/[Tween] TWEEN 20, plates were probed for 1 hour with monoclonal antibodies against mouse IgG subclasses IgG1, IgG2a, IgG2b, and IgG3 labeled with alkaline phosphatase obtained from Pharmingen, Inc., San Diego, CA. The optical density (O.D.) was measured at 405 nm.

Please replace the paragraph beginning on page 35, line15 with the following:

Affinity column chromatography using [HisBind] HISBIND resin was performed at 4°C according to manufacturer's specifications (Novagen, Madison, WI), except that all buffers were augmented with 6 M urea. Briefly, 5 ml of [HisBind] HISBIND resin in a 10 ml/glass column was washed with 15 ml of water, 25 ml of 50 mM $NiSO_4$ and 15 ml of binding buffer + urea to 6 M. The soluble fraction was passed over the resin and protein that bound nonspecifically was washed from the resin with 50 ml of binding buffer followed by 50 ml of washing buffer (20 mM Tris-HCl pH 7.9, 0.5 M NaCl, 60 mM imidazole) + urea to 6 M. The HisTag-Ipa fusion protein was then eluted from the column with elution buffer (20 mM Tris-HCl pH7.9, 0.5 M NaCl, 1 M imidazole) + urea to 6 M. At each step of the purification process, the concentration of protein in the sample was determined using the bicinchoninic acid (BCA) micro-assay (Sigma Chemical Co., St. Louis, MO) according to the manufacturer's instructions. The samples were stored at -20° C and the [HisBind] HISBIND resin was regenerated with 20 mM Tris-HCl pH 7.9, 0.5 M NaCl, 100 mM EDTA.

Please replace the paragraph beginning on page 37, line 3 with the following:

The recombinant SipC and IpaC fusion proteins described here contain a thrombin cleavage site at the junction between the target protein and its N-terminal HisTag leader. IpaC and SipC do not contain a thrombin cleavage site. Site-specific cleavage of each protein with thrombin yields a native IpaC or SipC protein product with two additional amino acids at its N terminus. After thrombin cleavage, the HisTag-containing leader peptide could be separated from the recombinant Ipa protein product by adding charged [HisBind] HISBIND resin to the mixture and lightly centrifuging to pellet the resin (along with the HisTag leader) while leaving the soluble Ipa protein in the supernatant. Thrombin cleavage efficiency approached completion using an overnight incubation at 20° C.

Please replace the paragraph beginning on page 11, line 19 with the following:

FIG. 1 shows a schematic of a linearized plasmid pET15b containing a DNA sequence encoding a recombinant invasin protein (IpaC or SipC). SEQ. ID. NO 19 (GAGACATATG) and SEQ. ID. NO. 20 (GGATCCGAGA) are also depicted.

AMENDMENTS TO THE CLAIMS

Listing of Claims:

Claims 1-12 cancelled.

13. (currently amended) A method for the production of a purified recombinant invasin protein comprising:

a) ~~inserting~~ preparing an expression vector comprising a polynucleotide encoding an invasin protein ~~into an expression vector~~;

b) transforming ~~the combination of a) into~~ a host cell with the expression vector;

c) growing the transformed host cell under conditions conductive to soluble invasin protein expression;

d) extracting the expressed invasin protein from a ~~host cell~~ lysate of the transformed host cell, a culture medium comprising the transformed host cell, or an ~~reconstituted~~ organism reconstituted from the transformed host cell with a solution comprising a protein denaturant;

e) performing an affinity purification of the extracted invasin protein ~~wherein the method of said purification is performed~~ in the presence of ~~a~~ the protein denaturant;

f) removing said protein denaturant from the purified invasin protein ~~solution obtained in the purification process of e)~~ until the concentration of the protein denaturant is at the minimum concentration necessary to maintain the solubility of the purified invasin protein ~~solubility~~; and

g) rapidly diluting the purified invasin protein into a volume of denaturant-free solution.

14. (currently amended) A method for the production of a purified recombinant invasin protein comprising:

a) ~~combining~~ preparing an expression vector comprising a polynucleotide encoding the invasin protein and a polynucleotide encoding an affinity purification moiety;

b) transforming ~~the combination of a), in an appropriate expression vector, into~~ a host cell with the expression vector;

c) growing the transformed host cell under conditions conducive to soluble invasin protein expression;

d) extracting the expressed invasin protein from a ~~host cell~~ lysate of the transformed host cell, a culture medium comprising the transformed host cell, or an ~~reconstituted~~ organism reconstituted from the transformed host cell with a solution comprising a protein denaturant;

e) performing an affinity purification of the extracted invasin protein ~~appropriate for the affinity purification moiety encoded by the polynucleotide in a), wherein the method of said purification is performed~~ in the presence of ~~a~~ the protein denaturant;

f) removing said protein denaturant from the purified invasin protein ~~solution obtained in the purification process of e)~~ until the concentration of the protein denaturant is at the minimum concentration necessary to maintain the solubility of the purified invasin protein ~~solubility~~; and

g) rapidly diluting the purified invasin protein into a volume of denaturant-free solution.

15. (original) The method of claim 14 wherein the affinity purification moiety is His-Tag.

16. (previously presented) The method of claim 14 wherein the protein denaturant is selected from the group consisting of guanidine hydrochloride, detergents, and urea.

17. (previously presented) The method of claim 14 wherein the protein denaturant is urea.

18. (original) The method of claim 17 wherein the concentration of urea is between about 1 M and about 10 M.

Claims 19-20 cancelled.

21. (currently amended) The method of claim 14 further comprising the step of removing the affinity purification moiety from the recombinant invasin protein after step e, f, or g.

22. (currently amended) The method of claim 14 wherein the dilution of the purified invasin protein occurs in about 1 minute or less.

Claims 23-24 cancelled.

25. (currently amended) A method for the production of a purified recombinant invasin protein comprising:

a) ~~combining~~ preparing an expression vector comprising a polynucleotide encoding the invasin protein and a polynucleotide encoding an affinity purification moiety;

b) transforming ~~the combination of a), in an appropriate expression vector, into~~ a host cell with the expression vector;

c) growing the transformed host cell under conditions conducive to soluble invasin protein expression;

d) extracting the expressed invasin protein from a ~~host cell~~ lysate of the transformed host cell, a culture medium comprising the transformed host cell, or an ~~reconstituted~~ organism reconstituted from the transformed host cell with a solution comprising 6 M urea;

e) performing an affinity purification of the extracted invasin protein ~~appropriate for the affinity purification moiety encoded by the polynucleotide in a), wherein the method of said purification is performed~~ in the presence of a protein denaturant;

f) removing said protein denaturant from the purified invasin protein ~~solution obtained in the purification process of e)~~ until the concentration of the protein denaturant is at the minimum concentration necessary to maintain the solubility of the purified invasin protein ~~solubility~~; and

g) diluting the purified invasin protein in about 10 seconds or less into a volume of denaturant-free solution.

Claims 26-100 cancelled.

101. (previously presented) The method of claim 13 wherein the protein denaturant is selected from the group consisting of guanidine hydrochloride, detergents, and urea.

102. (previously presented) The method of claim 13 wherein the protein denaturant is urea.

103. (currently amended) The method of claim 13 wherein the dilution of the purified invasin protein occurs in about 1 minute or less.

104. (new) The method of claim 103 wherein the dilution of the purified invasin protein occurs in less than 10 seconds.

105. (new) The method of claim 13 wherein the purified invasin protein refolds without forming insoluble aggregates.

106. (new) The method of claim 13 wherein the purified recombinant invasin protein comprises an amino acid sequence derived from an invasin protein of a bacterium chosen from the group consisting of *Shigella* spp., *Salmonella* spp., and enteroinvasive *E. coli*.

107. (new) The method of claim 106 wherein the purified recombinant invasin protein is an IpaC or a SipC protein.

108. (new) The method of claim 106 wherein the purified recombinant invasin protein comprises an amino acid sequence chosen from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2.

109. (new) The method of claim 13 wherein the purified recombinant invasin protein comprises an amino acid sequence derived from an invasin protein of a bacterium chosen from the group consisting of *Shigella* spp., *Salmonella* spp., and enteroinvasive *E. coli* and the dilution of the purified invasin protein occurs in about 1 minute or less.

110. (new) The method of claim 109 wherein the purified recombinant invasin protein is an IpaC or a SipC protein.

111. (new) The method of claim 109 wherein the purified recombinant invasin protein comprises an amino acid sequence chosen from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2.

112. (new) The method of claim 14 wherein the purified invasin protein refolds without forming insoluble aggregates.

113. (new) The method of claim 14 wherein the purified recombinant invasin protein comprises an amino acid sequence derived from an invasin protein of a bacterium chosen from the group consisting of *Shigella* spp., *Salmonella* spp., and enteroinvasive *E. coli*.

114. (new) The method of claim 113 wherein the purified recombinant invasin protein is an IpaC or a SipC protein.

115. (new) The method of claim 113 wherein the purified recombinant invasin protein comprises an amino acid sequence chosen from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2.

116. (new) The method of claim 14 wherein the purified recombinant invasin protein comprises an amino acid sequence derived from an invasin protein of a bacterium chosen from the group consisting of *Shigella* spp., *Salmonella* spp., and enteroinvasive *E. coli* and the dilution of the purified invasin protein occurs in about 1 minute or less.

117. (new) The method of claim 116 wherein the purified recombinant invasin protein is an IpaC or a SipC protein.

118. (new) The method of claim 116 wherein the purified recombinant invasin protein comprises an amino acid sequence chosen from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2.

119. (new) The method of claim 22 wherein the dilution of the purified invasin protein occurs in less than 10 seconds.

120. (new) The method of claim 25 wherein the purified recombinant invasin protein comprises an amino acid sequence derived from an invasin protein of a bacterium chosen from the group consisting of *Shigella* spp., *Salmonella* spp., and enteroinvasive *E. coli.*

121. (new) The method of claim 120 wherein the purified recombinant invasin protein is an IpaC or a SipC protein.

122. (new) The method of claim 120 wherein the purified recombinant invasin protein comprises an amino acid sequence chosen from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2.

REMARKS

Claims 1-7, 10, 12-18, 21, 22, 25, 26, 29-33, 38-44, 47, 51-56, 61-68, 70, 72, 73, 77-80, 82-87, 90-92, 94-96, and 98-103 are currently pending. New claims 104-122 have been added. Claims 1-7, 10, 12, 26, 29-33, 38-44, 47, 51-56, 61-68, 70, 72, 73, 77-80, 82-87, 90-92, 94-96, and 98-100 are cancelled as being directed to non-elected inventions. Applicants expressly reserve the right to file divisional applications directed to these non-elected claims in the future. Claims 13-14, 16-17, 21, 25, and 101-103 are amended herein and new claims 104-122 are added to more particularly claim the subject matter of the present invention.

Sequence Rule Compliance

The sequences found in Figure 1 have been added to the Sequence Listing as SEQ. ID. NOS. 19 and 20. In addition, the paragraph beginning on page 11, line 19 in the Brief Description of the Drawings has been amended to provide sequence identifiers for these sequences. Applicants note that under MPEP §2422.02, where a sequence is given in a Figure, the sequence identifier must be used in the drawing or in the Brief Description of the Drawings.

Objections to the Specification

The specification has been amended to capitalize the trademarks appearing on page 27, line 18; page 33, line 29; page 35, lines 15, 17, and 26; page 36, lines 15 and 16; page 37, line 8; and page 44, line 26 in compliance with MPEP §608.01(v).

Rejections under 35 U.S.C. §112, second paragraph

Reconsideration is requested of the rejection of claims 13-18, 21, 22, 25, and 101-103 under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicants regard as the invention.

(a) The Office has objected to the phrase "the combination of a)" in step b of claim 13 as having improper antecedent basis. The phrase "the combination of a)" has been deleted from step b, which now reads "transforming a host cell with the expression vector." Proper antecedent basis for the phrase "the expression vector" can be found in step a ("preparing an expression vector").

(b) Likewise, the Office also objects to the phrase "the combination of a)" in step b of claim 14. The phrase "the combination of a)" has been deleted from step b, which now reads "transforming a host cell with the expression vector." Proper antecedent basis for the phrase "the expression vector" can be found in step a.

(c) The Office has stated that it is unclear what characteristics an expression vector has to have in order to qualify as an "appropriate" expression vector in claims 14 and 25. The term "appropriate" has been deleted from the claims.

(d) The Office has objected to the use of the term "rapidly" in claims 13 and 14 as being indefinite. MPEP §2173.02 indicates that the definiteness of claim language must be analyzed, not in a vacuum, but in light of the content of the particular application disclosure. The meaning of "rapidly" is clear from the specification--rapid or "sudden dilution of the purified protein solution into a denaturant-free solution allow[s]

the protein to refold without forming insoluble aggregates."[1] The Specification also provides guidelines for determining the meaning of "rapidly."[2]

(e) Step c of claims 13, 14, and 25 has been amended to change "conditions conducive to soluble protein expression" to "conditions conducive to soluble invasin protein expression."

(f) The Office has rejected claims 13, 14, and 25 as vague, indefinite, and confusing in that the claims do not distinctly claim the subject matter which the applicants regard as the invention. In particular, the Office has objected to the phrase "extracting the protein from a host cell lysate, culture medium, or reconstituted organism."

The Office has objected to the phrase "a host cell" in step d. Step d of claims 13, 14, and 25 has been amended to change "a host cell lysate" to "a lysate of the transformed host cell." Proper antecedent basis for "the transformed host cell" may be found in step c of claims 13, 14, and 25.

The Office has also stated that it is unclear how the protein can be extracted from any "culture medium" or "a reconstituted organism," and unclear what is contained in the culture medium and what organism is reconstituted. Step d of claims 13, 14, and

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[1] Specification, p. 28, ln. 19-20. The Specification also states, "Without limiting the invention to any particular theory or mechanism, applicants believe that the rapid removal of denaturant allows beneficial protein *intra-actions*, necessary for correct protein folding, to occur while the rapid dilution of the protein solution minimizes the probability of detrimental protein-protein *interactions*, which form aggregates." *Id.* at ln. 20-24.

[2] "The invasin protein solution, containing the minimum concentration of denaturant, is then rapidly diluted into a buffer containing no denaturant. This process is preferably completed in less than one minute, more preferably in less than 30 seconds, and most preferably in less than 10 seconds." Specification, p. 29, ln. 1-4.

25 has been amended to change "culture medium" to "a culture medium comprising the transformed host cell"[3] and "a reconstituted organism" to "an organism reconstituted from the transformed host cell."[4] The amendments make clear what is contained in the culture medium and what organism is reconstituted. Antecedent basis for "the transformed host cell" can be found in step c of claims 13, 14, and 25.

(g) The phrase "wherein the method of said purification is performed" in step e of claims 13, 14, and 25 has been removed.

(h) Step f of claims 13, 14, and 25 has been amended to change "the denaturant" to "the protein denaturant."

(i) The Office has objected to the phrase "the protein solution" in step f of claims 13, 14, and 25 as having improper antecedent basis. Step f of claims 13, 14, and 25 has been amended to change "the protein solution" to "the purified invasin protein."

(j) The phrase "the purification process of e)" has been removed from step f of claims 13, 14, and 25.

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[3] As noted in the Specification, the host cell may be grown in culture and the invasin protein may be secreted into culture media. *See, e.g.*, Specification, Example 1. *See also* Specification, p. 26-27 (indicating transformed host cells may be grown in culture) and Specification, p. 27, ln. 14.

[4] As noted in the Specification, the protein may be expressed using plant or animal cells which have been reconstituted into whole organisms. Specification, p. 27, ln. 3-4 ("When utilizing plant or animal cells which have been reconstituted into whole organisms...").

(k) Step f of claims 13, 14, and 25 has been amended to change "protein solubility" to "the solubility of the purified invasin protein."

(l) Step g of claims 13, 14, and 25 has been amended to change "the purified protein" to "the purified invasin protein."

(m) Claims 22 and 103 have been amended to change "the purified protein" to "the purified invasin protein."

(n) The Office has stated that "denaturant" as used in the phrase "denaturant-free solution" in step g of claims 13, 14, and 25 is not clear. MPEP §2173.02 indicates that the definiteness of claim language must be analyzed, not in a vacuum, but in light of the content of the particular application disclosure. The specification clearly defines "denaturant" as "a chemical substance which induces a conformational change in a protein, interfering with protein-protein intra-actions and causing it to lose its tertiary structure. Examples of denaturants are urea and detergents."[5] One of ordinary skill in the art would thus understand what is meant by the phrase "denaturant-free solution," in light of the definition of "denaturant" provided by the specification.

(o) Claim 14, step e has been amended to change "a protein denaturant" to "the protein denaturant." Antecedent basis for the phrase "the protein denaturant" in claim 14, step e and in claims 16 and 17 is found in claim 14, step d.

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[5] Specification, p. 13, ln. 28-30.

(p) Claim 13, step e has been amended to change "a protein denaturant" to "the protein denaturant." Antecedent basis for the phrase "the protein denaturant" in claim 13, step e and in claims 101 and 102 is found in claim 13, step d.

(q) Claim 21 has been amended to add the phrase "after step e, f, or g" to clarify that the affinity purification moiety may be removed from the recombinant invasin protein after any of these steps.[6]

(r) The Office has objected to the use of the term "moiety" in claims 14 and 21 "because it is unclear what is contained in this limitation." As previously discussed, the definiteness of claim language must be analyzed in light of the content of the particular application disclosure.[7] The phrase actually used in claims 14 and 21 is "affinity purification moiety." The specification defines "affinity purification moiety" as "moiety that has been added to a protein in order to allow the protein to be purified using some affinity purification scheme."[8] Furthermore, the specification gives several examples of affinity purification systems that may be used.[9] One skilled in the art

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[6] Support for this amendment is found in the Specification, p. 28, ln. 4-5, which states that "After the protein has been purified, or at a later step, the affinity purification moiety may be selectively cleaved from the recombinant invasin protein..."

[7] MPEP §2173.02.

[8] Specification, p. 13, ln. 22-24. The specification further states, "This portion of the protein may or may not be cleaved from the protein after purification. An example of an affinity purification moiety is the poly-histidine nickel-chelating amino acid sequence described in U.S. Patent No. 5,594,115 and commercially available under the name His-Tag® (Novagen, Madison, WI)." *Id.* at ln. 22-27.

[9] For example, the specification describes affinity purification systems based on fusion proteins which contain metal chelating amino acid sequences, and purification systems using a peptide ligand of a ribonuclease, peptides recognized by specific bound antibodies, a peptide that binds to cellulose, or a peptide which binds to biotin *in vivo*, which then binds as a complex to a streptavidin-coated matrix. See Specification,

would thus understand what is meant by the phrase "affinity purification moiety" in light of the definition and examples provided by the specification.

(s) In light of the foregoing, applicants submit that claims 15-18, 21, 22, and 101-103 are not indefinite because of their dependency on claims 13 or 14.

Rejections under 35 U.S.C. §102(b)

Reconsideration is requested of the rejection of: (i) claims 13-18, 21, 22, and 101-103 under 35 U.S.C. §102(b) as being anticipated by Paul, et al.; (ii) claims 13-16, 21, 22, 101, and 103 under 35 U.S.C. §102(b) as being anticipated by Picking, et al.; and (iii) claims 13, 14, 16, 22, 101, and 103 under 35 U.S.C. §102(b) as being anticipated by Leong, et al.

Claim 13 is directed to a method for the production of a purified recombinant invasin protein. The method comprises: a) preparing an expression vector comprising a polynucleotide encoding an invasin protein; b) transforming a host cell with the expression vector; c) growing the transformed host cell under conditions conductive to soluble invasin protein expression; d) extracting the expressed invasin protein from a lysate of the transformed host cell, a culture medium comprising the transformed host cell, or an organism reconstituted from the transformed host cell with a solution comprising a protein denaturant; e) performing an affinity purification of the extracted invasin protein in the presence of the protein denaturant; f) removing said protein denaturant from the purified invasin protein until the concentration of the protein denaturant is at the minimum concentration necessary to maintain the solubility of the purified invasin protein; and g) rapidly diluting the purified invasin protein into a volume of denaturant-free solution.

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p. 20-23.

Claim 14 is directed to a method for the production of a purified recombinant invasin protein. The method comprises: a) preparing an expression vector comprising a polynucleotide encoding the invasin protein and a polynucleotide encoding an affinity purification moiety; b) transforming a host cell with the expression vector; c) growing the transformed host cell under conditions conducive to soluble invasin protein expression; d) extracting the expressed invasin protein from a lysate of the transformed host cell, a culture medium comprising the transformed host cell, or an organism reconstituted from the transformed host cell with a solution comprising a protein denaturant; e) performing an affinity purification of the extracted invasin protein in the presence of the protein denaturant; f) removing said protein denaturant from the purified invasin protein until the concentration of the protein denaturant is at the minimum concentration necessary to maintain the solubility of the purified invasin protein; and g) rapidly diluting the purified invasin protein into a volume of denaturant-free solution.

Claims 15-18, 21-22, 101-103, and new claims 104-119 are either directly or indirectly dependent on claims 13 or 14.

1. Paul, et al. (Human Gene Therapy, 8:1253-62 (July 1, 1997))

Paul, et al. describe the fusion of the GAL4 DNA-binding domain with the invasin cell binding, internalization domain of the *Yersinia pseudotuberculosis inv* gene product, invasin, via a flexible protein linker sequence to produce a protein that can be used to deliver DNA specifically to target cells. Paul, et al. describe growing E. coli containing plasmids encoding GAL4, Inv, or GAL4/Inv, and inducing protein expression. The cells were harvested by centrifugation, and cell pellets were resuspended in lysis buffer that contained 6 M guanidine-HCl, sonicated, and filtered. The cell lysates were passed over a HiTrap Chelating column that had been equilibrated in a buffer containing 8 M urea. Fusion proteins containing a hexahistidine tag were eluted. Prior to dialysis pooled fractions containing protein were diluted. The diluted pools were dialyzed against buffer 1 (which contained 6 M urea). The dialysis buffer was sequentially

diluted two-fold in buffer 1 (without urea) eight times at intervals of at least 2 hours. The samples were dialyzed against buffer 2 (which contained no urea or guanidine-HCl). The solutions were removed from the dialysis chamber and centrifuged. The soluble supernatant was decanted and analyzed by SDS-polyacrylamide gel electrophoresis. Protein concentrations were determined and final purity was found to be greater than 90%. After dialysis, 65-78% of the renatured protein was recovered in the soluble fraction after centrifugation.

Paul, et al. do not describe each and every element of claims 13 and 14.[10] Among other things, claims 13 and 14 both require removing the protein denaturant from the purified invasin protein until the concentration of the protein denaturant is at the minimum concentration necessary to maintain the solubility of the purified invasin protein (step f) and rapidly diluting the purified invasin protein into a volume of denaturant-free solution (step g). Paul, et al. disclose no such steps.

Although Paul, et al. make a general statement regarding empirically determining optimal dilutions to avoid aggregation during refolding, there is no mention of removing denaturant to a minimum concentration necessary to maintain solubility of the purified invasin protein, followed by rapid dilution of the purified invasin protein into a volume of denaturant-free solution. In contrast, Paul, et al. describe refolding the proteins by slowly and sequentially dialyzing away the denaturant.[11]

Furthermore, applicants respectfully disagree with the Office's assertion that "rapid" dilution into a denaturant-free solution is inherent in Paul, et al. In relying on inherency, the Office "must provide a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent feature necessarily

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[10] MPEP §2131 states that a claim is anticipated under 35 U.S.C. §102 only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference.

[11] *See* Paul, et al., p. 1255, col. 2.

flows from the teachings of the applied prior art."[12] In addition, the fact that a certain result or characteristic may occur or be present in the prior art is not sufficient for inherency.[13] No such showing has been made by the Office as to rapid dilution of purified invasin protein into a volume of denaturant-free solution. In fact, the only evidence supplied by the Office in support of inherency is a statement that the final recombinant invasin product remained soluble and biologically functional.

The protein renaturation process described by Paul, et al., as previously discussed, involves dialyzing the protein-containing samples against buffer 1 (which contained 6 M urea), and then sequentially diluting the dialysis buffer two-fold in buffer 1 (without urea) eight times at intervals of at least 2 hours. The samples are then dialyzed against buffer 2 (without urea or guanidine-HCl).[14] It is clear that Paul, et al. do not perform a rapid dilution into a denaturant-free solution, but rather perform a sequential dilution over the course of at least 16 hours.[15] Step g of claims 13 and 14 (i.e. rapidly diluting the purified invasin protein into a volume of denaturant-free solution) can thus not be said to be inherent in Paul, et al.[16]

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[12] MPEP §2112, citing *Ex parte Levy*, 17 USPQ2d 1461, 1464 (Bd. Pat. App. & Inter. 1990) (emphasis in original).

[13] See MPEP §2112, citing *In re Rijckaert*, 9 F.3d 1531, 1534 (Fed. Cir. 1993).

[14] Paul, et al., at p. 1255, col. 2.

[15] The sequential dilutions were performed eight times at intervals of at least 2 hours, i.e. a period of at least 16 hours.

[16] Applicants further note that the invasins described in Paul, et al. are from *Yersinia pseudotuberculosis*, and are thus different from the invasin proteins described in the present application. It can not therefore be assumed that the invasins of Paul, et al. would have the same characteristics as the invasins of the present invention. The purification strategy for the invasins of Paul, et al. would thus not necessarily be the same as for the invasin proteins of the present invention.

In light of the foregoing, applicants respectfully request withdrawal of the rejection of claims 13 and 14 under 35 U.S.C. §102(b) as being anticipated by Paul, et al. Claims 15-18, 21, 22, and 101-119 are either directly or indirectly dependent on claim 13 or 14 and are thus patentable for the same reasons as set forth above for these claims, as well as for the additional elements they require.

2. Picking, et al. (Protein Expression and Purification, 8:401-408 (1996))

Picking, et al. describe the cloning, expression, and purification of IpaB and IpaC fusion proteins from *E. coli*. More particularly, Picking, et al. attempted to improve the expression of soluble IpaC and IpaB. Picking, et al. prepared plasmids (pET32b) containing specific IpaB and IpaC gene fragments, an N-terminal leader containing thioredoxin, and six tandem histidine residues, and transformed *E. coli* with these plasmids. The *E. coli* were grown, and target protein synthesis was induced. The *E. coli* were collected by centrifugation, and the bacterial pellets were resuspended in HisBind binding buffer containing 0.1% Triton X-100 (v/v) and lysed by sonication. The soluble fraction was collected following centrifugation, and affinity column chromatography was performed using HisBind resin. The HisTag-Ipa fusion protein was eluted from the column with elution buffer, and dialyzed against a solution containing sodium phosphate, NaCl, and glycerol. Picking, et al. purified recombinant IpaB and IpaC to greater than 90% homogeneity using the nickel-chelation resin.

Picking, et al. do not describe each and every element of claims 13 and 14. Among other things, claims 13 and 14 both require removing the protein denaturant from the purified invasin protein until the concentration of the protein denaturant is at the minimum concentration necessary to maintain the solubility of the invasin protein (step f), and rapidly diluting the purified invasin protein into a volume of denaturant-free solution (step g). Picking, et al. disclose no such steps.

After affinity column chromatography, Picking, et al. elute the HisTag-Ipa fusion protein from the HisBind resin using elution buffer, and dialyze the eluted protein

against a solution containing sodium phosphate, NaCl, and glycerol. Picking, et al. do not describe step f; i.e., removing protein denaturant from the purified invasin protein until the concentration of the protein denaturant is at the minimum concentration necessary to maintain the solubility of the invasin protein.

Furthermore, applicants respectfully disagree with the Office's assertion that "rapid" dilution into a denaturant-free solution (i.e. step g) is inherent in Picking, et al. As previously discussed, when relying on inherency, the Office "must provide a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent feature necessarily flows from the teachings of the applied prior art."[17] No such showing has been made by the Office as to step g; the Office has merely asserted that rapid dilution into a denaturant-free solution is inherent in Picking, et al. because the final recombinant invasin protein product remained immunologically functional.

As noted in the specification, the rapid dilution into a denaturant free solution allows the protein to refold without forming insoluble aggregates.[18] Picking, et al. make no reference of this rapid dilution step, but rather, improve solubility by synthesizing fusion proteins with a thioredoxin leader. In fact, it is clear from Picking, et al. that it is the presence of the thioredoxin leader that imparts solubility to and promotes proper

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[17] MPEP §2112, citing *Ex parte Levy*, 17 USPQ2d 1461, 1464 (Bd. Pat. App. & Inter. 1990) (emphasis in original). The fact that a certain result or characteristic may occur or be present in the prior art is not sufficient for inherency. See MPEP § 2112, citing *In re Rijckaert*, 9 F.3d 1531, 1534 (Fed. Cir. 1993).

[18] Specification, at p. 28, ln. 18-20.

folding of the invasin protein purified therein.[19] Step g of claims 13 and 14 can thus not be said to be inherent in Picking, et al.

In light of the foregoing, applicants submit that claims 13 and 14 are not anticipated by Picking, et al. Claims 15-16, 21-22, 101, and 103-119 depend either directly or indirectly from claim 13 or 14, and are thus patentable for the same reasons as set forth above for claims 13 and 14, as well as for the additional elements they require.

3. Leong, et al. (EMBO J., 9:1979-89 (1990))

Leong, et al. describe the identification of the region of the invasin protein of *Yersinia pseudotuberculosis* responsible for cell recognition. Leong, et al. further describe the production and purification of invasin deletion derivatives (InvΔ53C and InvΔ254). Cells containing plasmids encoding invasin deletion derivatives were grown, and lysed in a French pressure cell. Unlysed cells were removed by centrifugation, and membranous debris was collected by a second round of centrifugation. The conditions used by Leong, et al. resulted in aggregation of approximately 70% of the overproduced carboxyl-terminal fragment of invasin. The pellet containing the aggregated invasin fragment was washed by resuspension in buffer followed by centrifugation, and the aggregated invasin was dissolved by resuspending the pelleted debris in a solution containing 6 M guanidine-HCl. The guanidine-HCl extract was sequentially dialyzed against a solution containing 0.5 M guanidine-HCl, followed by dialysis against a

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[19] "Use of the pET32b vector allows fusion of the Ipa proteins to a leader protein that is highly soluble in the *E. coli* cytoplasm [ ] and thus can act as a chaperone of sorts in promoting proper folding and enhancing the solubility of recombinant fusion proteins." Picking, et al., at p. 404-405. Picking, et al. further state, "To further improve expression of IpaC and IpaB, the gene for each was subcloned into pET32b for synthesis with an N-terminal thioredoxin leader. Thioredoxin leader proteins have been shown to confer a high degree of solubility to fusion proteins [ ] by acting as a chaperone of sorts in enhancing their proper folding." *Id.* at p. 406.

solution containing no guanidine-HCl. The soluble fraction containing invasin was concentrated by precipitation in 35% ammonium sulfate, and the resuspended pellet was dialyzed against a solution containing Tris-HCl and NaCl. Further purification was achieved by loading the dialysate onto a DEAE-cellulose column. The eluted fraction containing the invasin derivatives was then collected.

Leong, et al. do not describe each and every element of claims 13 and 14. Among other things, Leong, et al. do not disclose steps c, e, f, or g of claims 13 and 14.

For instance, step c of claims 13 and 14 calls for growing the transformed host cell under conditions conducive to soluble invasin protein expression. Leong, et al. do not use such conditions since, as previously discussed, the conditions used by Leong, et al. to overproduce the carboxyl-terminal fragment of invasin resulted in approximately 70% of the invasin in aggregates.[20] The aggregated invasin of Leong, et al. then had to be dissolved using 6 M guanidine-HCl.

In addition, step e of claims 13 and 14 calls for performing an affinity purification of the extracted invasin protein in the presence of a protein denaturant. In contrast, Leong, et al. merely describe loading a dialysate containing the invasin onto a DEAE-cellulose column equilibrated with Tris-HCl and NaCl, and eluting with a 50-250 mM linear NaCl gradient.[21] Thus, Leong, et al. do not use a protein denaturant during affinity purification.

Step f of claims 13 and 14 calls for removing the protein denaturant from the purified invasin protein until the concentration of the protein denaturant is at the minimum concentration necessary to maintain the solubility of the invasin protein.

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[20] *See* Leong, et al., at p. 1987, col. 1. Conditions conducive to soluble invasin protein expression are discussed on pages 26-27 of the Specification.

[21] Leong, et al., at p. 1987, col. 1.

Leong, et al. describe no such step.[22] Furthermore, such a step would not be necessary in the process described in Leong, et al. since, as discussed above, Leong, et al. do not use a protein denaturant during affinity purification.

Step g of claims 13 and 14 calls for rapidly diluting the purified invasin protein into a volume of denaturant-free solution. Such a step is not disclosed in Leong, et al. Furthermore, applicants respectfully disagree with the Office's assertion that the rapid dilution of purified invasin is inherent from the teachings of Leong, et al. The Office has not provided a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent feature necessarily flows from the teachings of the applied prior art;"[23] but has merely states that the final recombinant invasin protein product of Leong, et al. remained biologically functional. Furthermore, applicants respectfully note that it would be unnecessary to rapidly dilute the purified invasin derivative of Leong, et al. into a denaturant-free solution since, as previously discussed, Leong, et al. do not use a protein denaturant during purification on the DEAE-cellulose column (i.e., the invasin is already in a denaturant free solution). Step g of claims 13 and 14 can thus not be said to be inherent in Leong, et al.[24]

In light of the foregoing, applicants respectfully submit that claims 13 and 14 are not anticipated by Leong, et al. Claims 16, 22, 101, and 103-119 depend either directly

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[22] Leong, et al. merely state that after the invasin is eluted from the DEAE-cellulose column, "[t]he fractions containing the invasin derivative were pooled and stored frozen." *Id.*

[23] MPEP §2112, citing *Ex parte Levy*, 17 USPQ2d 1461, 1464 (Bd. Pat. App. & Inter. 1990) (emphasis in original). The fact that a certain result or characteristic may occur or be present in the prior art is not sufficient for inherency. See MPEP § 2112, citing *In re Rijckaert*, 9 F.3d 1531, 1534 (Fed. Cir. 1993).

[24] Applicants further note that the invasins described in Leong, et al. are from *Yersinia pseudotuberculosis*, and are thus different from the invasin proteins described in the present application. As discussed above, it can not therefore be assumed that the purification strategy for these invasins would necessarily be the same as for the invasin proteins of the present invention.

or indirectly from claims 13 or 14 and are thus patentable for the same reasons as set forth above for claims 13 and 14, as well as for the additional elements they require.

Rejections under 35 U.S.C. §103(a)

Reconsideration is requested of the rejection of claim 25 under 35 U.S.C. §103(a) as being unpatentable over Oaks, et al. (Clin. Diagnost. Lab. Immunol., 3:242-245 (1996)) in view of Comb, et al. (U.S. Patent No. 5,834,247), and/or Anilionis, et al. (U.S. Patent No. 5,192,338), Thorne (U.S. Patent No. 5,552,294), and Seed (U.S. Patent No. 5,726,293).

Claim 25 is directed to a method for the production of a purified recombinant invasin protein. The method comprises: a) preparing an expression vector comprising a polynucleotide encoding the invasin protein and a polynucleotide encoding an affinity purification moiety; b) transforming a host cell with the expression vector; c) growing the transformed host cell under conditions conducive to soluble invasin protein expression; d) extracting the expressed invasin protein from a lysate of the transformed host cell, a culture medium comprising the transformed host cell, or an organism reconstituted from the transformed host cell with a solution comprising 6 M urea; e) performing an affinity purification of the extracted invasin protein in the presence of a protein denaturant; f) removing said protein denaturant from the purified invasin protein until the concentration of the protein denaturant is at the minimum concentration necessary to maintain the solubility of the purified invasin protein; and g) diluting the purified invasin protein in about 10 seconds or less into a volume of denaturant-free solution.

Oaks, et al. describe the overexpression of a fusion protein consisting of full-length IpaD fused to a leader peptide containing six histidine residues. Oaks, et al. inserted an *ipaD* fragment into the plasmid expression vector pET-15b, and transformed *E. coli* with the plasmid. Overexpression of the *ipaD* fusion gene was induced. The fusion protein was affinity purified by passing a cytosolic extract

containing the recombinant protein over a Ni^{2+} column followed by elution of the IpaD fusion protein with 1 M imidazole.

For a combination of references to render obvious a claimed invention, the Office must show: (1) some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings; (2) a reasonable expectation of success; and (3) that the prior art reference (or references when combined) teach or suggest all the claim limitations.[25] The Office has not made such a showing.

Initially, applicants note that Oaks, et al. do not describe the use of a protein denaturant during the production and purification of the IpaD, and therefore do not disclose steps d to g of claim 25. Although Oaks, et al. do describe the use of imidazole as an eluant after affinity purification of the recombinant IpaD, applicants respectfully submit that imidazole is not a protein denaturant, as defined in the Specification. The Specification defines denaturant as "a chemical substance which induces a conformational change in a protein, interfering with protein-protein int**ra**-actions and causing it to lose its tertiary structure. Examples of denaturants are urea and detergents."[26] Imidazole is not such a denaturant.

The Office states that the imidazole used in Oaks, et al. qualifies as a protein denaturant in light of Seed, which teaches imidazole to be a mild denaturant. Seed discloses a method for eluting proteins from affinity matrices, and describes imidazole as a mild denaturant which disrupts protein int**er**actions, thereby facilitating the release of proteins bound to affinity ligands.[27] However, Seed does not describe imidazole as being able to induce a conformational change in a protein, interfere with protein-protein int**ra**-actions, or cause a protein to lose its tertiary structure; Seed merely uses

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[25] MPEP §2142.

[26] Specification, p. 13, ln. 28-30 (emphasis added).

[27] U.S. Patent No. 5,726,293, at col. 2, ln. 46-50.

imidazole as an elution reagent to release a protein from an affinity complex during affinity purification.[28]

One skilled in the art reading Oaks, et al. and Seed, would thus not consider imidazole to be a protein denaturant, as used in claim 25. In fact, in the absence of a denaturant such as guanidine or urea, elution of proteins from a purification matrix with imidazole is considered by those skilled in the art to be elution under non-denaturing conditions.[29] Thus, one skilled in the art would consider the production and purification of the recombinant IpaD in Oaks, et al. to be under non-denaturing conditions. Oaks, et al. can not, therefore, be said to describe steps d to g of claim 25.

Furthermore, there is no suggestion or motivation to combine Oaks, et al. with Seed, Comb, et al., Anilionis, et al., and/or Thorne to arrive at claim 25. Since Oaks, et al. do not use a protein denaturant in the production and purification of the IpaD, it therefore follows that no refolding steps would be required in the method described by Oaks, et al. There is thus no motivation in Oaks, et al. for one skilled in the art to modify the method described therein by using a protein denaturant or by performing steps f and g of claim 25. Furthermore, neither Seed, Comb, et al., Anilionis, et al., or Thorne provide such motivation or suggest that imidazole is a protein denaturant (as defined in the Specification).

Thorne merely discloses a method for detecting a virulence-associated factor (VAF) in a sample. The sample is contacted with a VAF releasing solution under conditions which release VAF, and the released VAF is immunochemically detected.

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[28] *Id.* at col. 3, ln. 11-14.

[29] For example, the Novagen product brochure, in describing purification on a HisBind Resin states: "After unbound proteins are washed away, the target protein is recovered by elution with imidazole...The versatile system allows proteins to be purified under gentle, non-denaturing conditions or in the presence of either 6M guanidine or urea." Novagen product brochure, at p. 2 (emphasis added). A Supplemental Information Disclosure Statement submitting the brochure is filed simultaneously herewith.

The VAF may be a surface antigen for invasin (Shigella dysenteriae or invasive *E. coli*),[30] and the immunological enhancing agent may be urea. The preferred concentration range of immunological enhancing agent is from about 0.1M to about 15M.[31]

Anilionis, et al. describe proteins and peptides related to an outer membrane protein of *Haemophilus influenzae*, and recombinant vectors containing nucleotide sequences encoding PBOMP-1 and PBOMP-2 related peptides, proteins and fusion proteins. Anilionis, et al. also describe the solubilization of a PBOMP-2:PBOMP-1 fusion protein using a solution containing 6M urea, and the removal of the urea after solubilization by dialysis against a buffer without urea.[32]

Comb, et al. disclose modified proteins comprising a controllable intervening protein sequence (CIVPS), which is capable of splicing or cleaving the target protein. The CIVPS may be used in purification of the target protein. Comb, et al. describe the use of 6M urea to solubilize a recombinant protein, and the rapid dilution of the proteins to allow refolding of the diluted proteins.[33] Although Comb, et al. do describe the purification of a recombinant protein using a Ni^{2+} affinity resin in the presence of 6M urea,[34] the protein purified in Comb, et al. is not an invasin protein, as described in the present application.

In light of the foregoing, applicants submit that claim 25 is not unpatentable over Oaks, et al. in view of Comb, et al. and/or Anilionis, et al., Thorne, and Seed. New claims 120-122 are either directly or indirectly dependent on claim 25 and are thus

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[30] U.S. Patent No. 5,552,294, at col. 4, ln. 14-15.

[31] *Id.* at col. 7, ln. 60-65.

[32] U.S. Patent No. 5,196,338, at col. 63, ln. 34-40.

[33] U.S. Patent No. 5,834,247, at col. 46-47.

[34] *See, e.g., id.* at col. 46, ln. 54-59.

patentable for the same reasons as set forth above for claim 25 as well as for the additional elements they require.

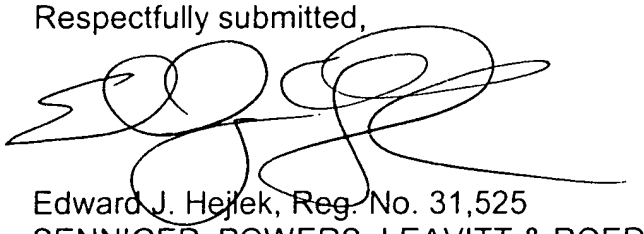
CONCLUSION

In light of the foregoing, applicants respectfully request reconsideration and withdrawal of the rejection of claims 13-18, 21, 22, 25, and 101-103 under 35 U.S.C. 112, second paragraph, the rejection of claims 13-18, 21, 22, and 101-103 under 35 U.S.C. §102(b), and the rejection of claim 25 under 35 U.S.C. §103(a).

Applicants also note that a signed copy of page 1 of applicants' IDS (listing references 1-11) was not included with the signed copy of pages 2-4, and therefore request the Office forward a signed copy of page 1.

Applicants further note that no shortened statutory period for response was indicated in the present office action. Therefore, applicants believe that this reply is timely, having been mailed prior to the end of the six month period for response. Furthermore, applicants believe that no extension fee is due, as no shortened statutory period was indicated. Nonetheless, should applicants' position be incorrect, the Commissioner is hereby authorized to charge any underpayment to Deposit Account No. 19-1345.

Respectfully submitted,



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EJH/cms

UOK 5320.1
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of William D. Picking, et al.
Serial No. 09/830,026
Filed October 20, 2001
Confirmation No. 9340
For: METHOD FOR THE PRODUCTION OF PURIFIED INVASIN PROTEIN AND USE THEREOF

May 21, 2004

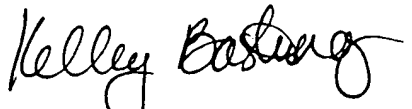
STATEMENT UNDER 37 C.F.R. 1.821(f)

TO THE COMMISSIONER FOR PATENTS,

SIR:

In accordance with 37 C.F.R. 1.821(f), I hereby state that the information recorded in computer readable form is identical to the written sequence listing submitted in support of the present application.

Respectfully submitted,



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<110> University of Kansas Center for Research
Walter Reed Army Institute for Research

<120> METHODS FOR THE PRODUCTION OF PURIFIED INVASIN PROTEIN AND USE THEREOF

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<141> 2001-10-20

<150> PCT/US99/24931
<151> 1999-10-21

<160> 20

<170> PatentIn version 3.1

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<212> PRT
<213> Salmonella typhimurium

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Leu Gly Leu Ser Pro Thr Leu Ser Ala Pro Ala Pro Gly Val Leu Thr

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Gln Thr Pro Gly Thr Ile Thr Ser Ser Leu Lys Ala Ser Ile Gln Asn
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Lys Ala Asn Glu Val Val Gln Thr Gln Leu Arg Glu Gln Gln Ala Glu
100 105 110

Val Gly Lys Phe Phe Asp Ile Ser Gly Met Ser Ser Ser Ala Val Ala
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Leu Leu Ala Ala Ala Asn Thr Leu Met Leu Thr Leu Asn Gln Ala Asp
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Ser Lys Leu Ser Gly Lys Leu Ser Leu Val Ser Phe Asp Ala Ala Lys
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Ser Ile Ser Gln Ser Ala Leu Gln Leu Gly Ile Thr Gly Val Gly Ala
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Asn Val Leu Asn Gly Gln Asn Ser Val Lys Leu Gly Ala Glu Gly Val
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Lys Ile Leu Met Met Arg Arg Leu Asn Leu Met Pro Glu Pro Ala Pro
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Ser Ser Ser Glu Thr Gln Lys Ser Gln Asn Tyr Gln Gln Ile Ala Ala
35 40 45

His Ile Pro Leu Asn Val Gly Lys Asn Pro Val Leu Thr Thr Thr Leu
50 55 60

Asn Asp Asp Gln Leu Leu Lys Leu Ser Glu Gln Val Gln His Asp Ser
65 70 75 80

Glu Ile Ile Ala Arg Leu Thr Asp Lys Lys Met Lys Asp Leu Ser Glu
85 90 95

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Met Ser His Thr Leu Thr Pro Glu Asn Thr Leu Asp Ile Ser Ser Leu
            100                 105                 110

Ser Ser Asn Ala Val Ser Leu Ile Ile Ser Val Ala Val Leu Leu Ser
        115                 120                 125

Ala Leu Arg Thr Ala Glu Thr Lys Leu Gly Ser Gln Leu Ser Leu Ile
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Ile Thr Gly Ile Gly Ala Lys Lys Thr His Ser Gly Ile Ser Asp Gln
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Lys Gly Ala Leu Arg Lys Asn Leu Ala Thr Ala Gln Ser Leu Glu Lys
        195                 200                 205

Glu Leu Ala Gly Ser Lys Leu Gly Leu Asn Lys Gln Ile Asp Thr Asn
    210                 215                 220

Ile Thr Ser Pro Gln Thr Asn Ser Ser Thr Lys Phe Leu Gly Lys Asn
225                 230                 235                 240

Lys Leu Ala Pro Asp Asn Ile Ser Leu Ser Thr Glu His Lys Thr Ser
                245                 250                 255

Leu Ser Ser Pro Asp Ile Ser Leu Gln Asp Lys Ile Asp Thr Gln Arg
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Arg Thr Tyr Glu Leu Asn Thr Leu Ser Ala Gln Gln Lys Gln Asn Ile
        275                 280                 285

Gly Arg Ala Thr Met Glu Thr Ser Ala Val Ala Gly Asn Ile Ser Thr
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Ser Gly Gly Arg Tyr Ala Ser Ala Leu Glu Glu Glu Glu Gln Leu Ile
305                 310                 315                 320

Ser Gln Ala Ser Ser Lys Gln Ala Glu Glu Ala Ser Gln Val Ser Lys
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Glu Ala Ser Gln Ala Thr Asn Gln Leu Ile Gln Lys Leu Leu Asn Ile
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UOK 5320.1
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of William D. Picking et al. Art Unit 1642
Serial No. 09/830,026
Filed October 20, 2001
Confirmation No. 9340
For METHOD FOR THE PRODUCTION OF PURIFIED INVASIN PROTEIN AND USE THEREOF

May 21, 2004

COMMISSIONER FOR PATENTS
P.O. BOX 1450
ALEXANDRIA, VIRGINIA 22313-1450

SIR:

INFORMATION DISCLOSURE STATEMENT

In accordance with 37 C.F.R. 1.97 and 1.98 and MPEP 609, and in compliance with the duty of disclosure set forth in 37 C.F.R. 1.56, applicant submits copies of the * references listed on the attached PTO/SB/08A for consideration by the Patent and Trademark Office in the above-entitled application and to be made of record therein.

* A check in the amount of $180.00 is enclosed to cover the fee specified in 37 CFR §1.17(p) for submission of this Information Disclosure Statement. The Commissioner is hereby authorized to charge any underpayment and credit any overpayment of government fees to Deposit Account No. 19-1345.

Respectfully submitted,

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EJH/cms
Express Mail Label No. EV 416452675 US

PTO/SB/08A

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(use as many sheets as necessary)

Complete if Known	
Application Number	09/830,026 JUL 09 2004
Filing Date	October 20, 2001
Confirmation Number	9340
First Named Inventor	William D. Picking
Group Art Unit	1642
Examiner Name	S. Devi
Attorney Docket No.	UOK 5320.1

Sheet	1	of	1

OTHER ART - NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.) date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[6]
	32.	Novagen Product Brochure, "His Bind® Kits", 2003, page 2	

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1]Unique citation designation number. [2]See attached Kinds of U.S. Patent Documents. [3]Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4]For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5]Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST. 16 if possible. [6]Applicant is to place a check mark here if English language Translation is attached or place an "A" here if English language abstract is attached..

SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

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* patent pending

United States & Canada 800-207-0144
Germany 0800 6931 000
United Kingdom 0800 622935
Or your local sales office
www.novagen.com

About the Kits

Product	Size	Cat. No.
His•Bind Resin	10 ml	69670-3
	50 ml	69670-4
	100 ml	69670-5
His•Bind Columns	pkg/5	70971-3
	pkg/25	70971-4
His•Mag™ Agarose Beads	2 ml	71002-3
	10 ml	71002-4
His•Bind Buffer Kit	1 kit	69755-3
His•Bind Purification Kit	1 kit	70239-3
His•Bind Quick Columns	pkg/12	70159-3
	pkg/60	70159-4
His•Bind Quick 300 Cartridges	pkg/10	70155-3
	pkg/50	70155-4
His•Bind Quick 900 Cartridges	pkg/10	70156-3
	pkg/50	70156-4
His•Bind Quick Buffer Kit	1 kit	70665-3
His•Bind Fractogel® (S)	25 ml	70692-3
His•Bind Fractogel (M)	25 ml	70693-3
BugBuster® His•Bind Purification Kit	1 kit	70793-3
PopCulture™ His•Mag Purification Kit	1 kit	71114-3

Description

His•Bind Resin and Buffer Kit

His•Bind Resin is used for rapid one-step purification of proteins containing a His•Tag® sequence by metal chelation chromatography. The His•Tag sequence binds to Ni^{2+} cations, which are immobilized on the His•Bind resin using the Charge Buffer supplied in the His•Bind Buffer Kit. After unbound proteins are washed away, the target protein is recovered by elution with imidazole. The His•Bind Resin can be regenerated and reused many times. The versatile system allows proteins to be purified under gentle, non-denaturing conditions or in the presence of either 6 M guanidine or urea. Up to 20 mg of target protein can be purified on a single 2.5-ml column.

His•Bind Columns

His•Bind Columns are packed with 1.25 ml of Ni^{2+}-charged His•Bind resin. The binding capacity is 10 mg of target protein per column. Top and bottom frits ensure even buffer flow and minimal disturbance of the bed when loading and running the column. The His•Bind Columns can be used under native conditions or in the presence of either 6 M guanidine or urea. Reuse is not recommended.

His•Mag Agarose Beads

His•Mag Agarose Beads are 3 µ diameter beads pre-charged with Ni^{+2}, and are ideal for rapid purification of multiple samples with minimal handling. The beads are compatible with magnetic separation based high throughput applications. Proteins can be purified under non-denaturing conditions or in the presence of either 6 M guanidine or urea.

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Germany 0800 6931 000
United Kingdom 0800 622935
Or your local sales office
www.novagen.com